ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

IMPROVING THE WATER SOLUBILITY OF CURCUMIN

M.Sc. THESIS

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Department of Chemistry

Chemistry

APRIL 2015

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FOREWORD

I would like to thank my family, my mother Ayla Katmer, my father Rafet Katmer and my sister Esra Katmer for all their support during my educational life. I also want to thank my advisor Prof. Dr. Giz for her guidance both in my bachelor and master studies.

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SYMBOLS AND ABBREVIATIONS

g : gram

mg : milligram

L : liter

nm : nanometer

UV-Vis : Ultraviolet – Visible

PEG : Poly(ethylene) glycol

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IMPROVING THE WATER SOLUBILITY OF CURCUMIN

SUMMARY

This thesis is about an alternative method for increasing the low water solubility of curcumin, which is a very useful antioxidant for human body.

Human body may produce some free radicals naturally whereas the free radicals may also be produced because of external effects like smoking cigarettes or consuming highly toxic dietary products in daily life.These free radicals are especially harmfull for human body cells and even destroy the DNA. For neglecting any kind of destruction,the free radicals produced by either humabn body or external effects,should be neutralized by antioxidants.

Curcumin is one of the most important antioxidants,extracted from the turmeric plant.It is very useful for treating certain diseases like cancer. There are many different types of pharmaceutical and alternative medicine products which contain curcumin as the main ingredient.

The water solubility of curcumin, is not sufficient for many of its pharmaceutical uses. Since the human body consists of mostly water, it is easier to consume a highly water soluble antioxidant. Low water solubility limits the curcumin applications in drug and foods. In this study, polyethyleneglycol is chosen as complexing agent for increasing the water solubility and it is bicompatible and not toxic. It is a non-ionic surfactant, which has an advantage that is the solvating power for numerous substances that are sparingly soluble in water. This action can be described to the formation of a sort of complex between the polyethylene glycol and the active substance. Different molecular weights from 400 which is a nonvolatile liquid, to 4000-5000 which is waxy at room temperature, are available. Three types of Polyethyleneglicol with different molecular weights were used for the experiments.

Three different PEG concentrations were chosen and PEG solutions were prepared. Since the curcumin is a coloured reagent, it was known that it will give a maximum absorbance at a specific wavelength. The measurements are made with a UV Visible dedector in a range of 300 to 500 nm. Three different curcumin concentrations are chosen having PEG and curcumin as the main component. For each concentration, three set of samples are prepared. Each set of sample solutions are also measured with the same method as the calibration solutions. The absorbance values at the maximum wavelength are obtained so that the amount of dissolved curcumin in PEG can be calculated via using the calibration equations.

As we can see from the results of the studies, curcumin amounts in the PEG solutions were increased in high concentrated PEG solutions and especially high molecular weight PEG types. PEG1500 solutions have the highest solubility of the curcumin. Although it is expected to have a highe solubility value in 20% of PEG1500, higher curcumin concentration is obtained in 10 and 15%.

KURKUMİNİN SUDAKİ ÇÖZÜNÜRLÜĞÜNÜN ARTTIRILMASI

ÖZET

Bu tez, insan vücudu için çok önemli bir antioksidan olan kurkumin maddesinin düşük değerlerdeki suda çözünürlüğünü arttırmaya yönelik olası alternatif methodlar üzerine çalışmalar içermektedir.

İnsan vücudu iç veya dış kaynaklı olarak çeşitli sebeplerle serbest radikaller üretebilir. Serbest radikal oluşumu bir atomun elektron alması ya da elektron vermesi olarak tanımlanabilir. Bu serbest radikallerin oluşum sebepleri arasında sigara içmek,toksin içerikli gıdaları tüketmek ve bunlar gibi birçok sebep yer alır. Vücutta oluşan serbest radikaller nötralize edilmedikleri takdirde insan vücudundaki birçok hücreye zarar verebilmektedirler.Serbest radikallerin zararlarından birisi insanın DNA'sına etki etmektedir.DNA üzerindeki bu zarar,kalıcı ve ciddi sonuçlara sebep olabilmektedir.

Vücutta oluşan serbest radikallerin gerek vücut tarafından üretilen gerek dışarıdan alınan bir takım maddelerle nötralize edilmesi gerekmektedir. Serbest radikallerin nötralize edilmesi onların vücuda zarar vermelerinin engellenmesi anlamına gelmektedir.Serbest radikaller nötralize edildikten sonra hücrelere zarar verme olasılıkları düşer.Serbest radikallerin nötralize edilmesinde insan vücudu kendi kendine antioksidanlar üreterek serbest radikalleri nötralize edebilen bir mekanizmaya sahiptir. Ancak vücudun ürettiği antioksidanların yanısıra vücutta barındırılan tüm potansiyel zararlı serbest radikallerin nötralize edilebilmesi için dışarıdan da antioksidan alımı gerekmektedir.

Vücuda dışarıdan alınan antioksidanlar genelde gıdalar aracılığıyla alınmaktadırlar. Gıda ile alınabilecek antioksidanlar için birçok örnek verilebilir.Vücuda dışarıdan alınarak serbest radikalleri nötralize edebilecek doğal antioksidanlardan birisi de kurkumin denen,turmerik bitkisinden ekstrakte edilen,Hindistan kökenli bir maddedir. Kurkumin keskin koyu sarı renge sahip,yiyeceklere renk ve tat vermek adına gıda sektöründe de sıkça kullanılan bir antioksidandır. Kurkuminin insan sağlığı açısından birçok faydası bulunduğu gibi aynı zamanda E100 kod ismiyle gıdalara eklenen maddeler listesinde de güvenli olarak sınıflandırılmaktadır. Günümüzde Hindistan başta olmak üzere birçok Asya ülkesinde geniş alanlarda tüketilen kurkumin, zerdeçal bitkisi olarak da nitelendirilebilmektedir.Kurkumin başta kanser olmak üzere çok sayıda ciddi hastalığın tedavisinde çok önemli bir etkiye sahiptir. İçerisinde aktif madde olarak kurkumini içeren birçok farklı çeşitte ilaç ve alternatif tıp ürünü bulunmaktadır.

İnsan vücuduna faydalı olmasına ve serbest radikalleri nötralize edebilecek yüksek antioksidan özelliğinesahip olmasına rağmen kurkuminin sudaki çözünürlüğü beklenenden azdır. İnsan vücudunun büyük kısmı sudan oluştuğu için,suda çözünürlüğü yüksek olan antioksidanları tüketmek insanlar için daha kolaydır. Dolayısıyla sudaki çözünürlüğünün düşük olması sebebiyle kurkumin insan

vücudunda zor çözünür Kimyasal maddeler için en önemli fiziksel özelliklerden birisi olan suda çözünürlük konusunda kurkumin yeterli olmadığından bu özelliğinin geliştirilmesi gerekmektedir.

Bu tezdeki çalışmalarda kurkuminin sudaki çözünürlüğünü arttırılması hedeflenmiştir.Kurkuminin sudaki çözünürlüğünü arttırabilmek için,kurkuminin seçilen diğer bir reaktifle kompleks oluşturarak çözünürlüğünün arttırılmasını içeren çalışmalar vardır ve bu tezde de yöntem olarak bu yöntem seçilmiştir. Bu yöntem dahilinde dikkat edilmesi gereken noktalardan birisi kurkumin ile kompleks oluşturarak maddenin sudaki çözünürlüğünü arttırması hedeflenen kompleks yapıcı reaktifin insan sağlığı açısından hiçbir zarara sahip olmamasıdır.Aynı zamanda bu kompleks yapıcı reaktif,kurkuminin bünyesinde barındırdığı insan için faydalı olan antioksidan özelliklerini de bozmayan bir kompleks oluşturabilmesi gerekmektedir.

Bu çalışmada kompleks yapıcı reaktif olarak polietilen glikol adı verilen bir polimer madde seçilmiştir. Polietilenglikol, etilen oksit monomerlerinin su gibi bir başlangıç materyali ile polimerizasyonu sonucunda elde edilebilen bir polimerdir. Polimerizasyon esnasında istenilen molekül ağırlığına ulaşıldığında reaksiyon durdurulabilir.Polietilenglikolün 400'e yakın sayıda farklı ortalama molekül ağırlığına sahip çeşitleri vardır ve bunlar oda sıcaklığında uçucu değillerdir. Polietilenglikol polimerinin gıda sektöründeki kullanım alanı da oldukça geniştir.Gıda tüketim ürünlerinde kullanılan polietilenglikolüninsan vücudu için günlük tüketim miktarı Dünya Sağlık Örgütü tarafından 10mg/kg olarak belirtilmiştir.Polietilenglikol, non iyonik bir surfaktandır.Bu sebeple polietilenglikol non iyonik bir surfaktan olarak suda çözünürlüğü çok zor olan maddelerin sudaki çözünürlüğünü arttırma gibi bir avantaja sahiptir. Bu olay polietilenglikol ile aktif maddenin arasında bir kompleks çeşidi oluşturulması olarak tanımlanabilir. Dolayısıyla oluşan kompleksin sudaki çözünürlüğünün yüksek olmasından faydalanılarak esas maddenin de bu özelliğinin iyileştirilmesi mümkündür.

Bu tezdeki çalışmalarda kurkumin ile seçilen bir reaktif arasında farklı konsantrasyonlardaki çözeltilerin hazırlanması,bu çözeltilerin spektrofotometrik yöntemlerle ölçümlenmesi ve kurkuminin sudaki çözünürlüğünün arttırılması çalışmaları gerçekleştirilmiştir. Seçilen kompleks oluşturucu reaktif polietilenglikoldür. Bu maddenin suda çözünürlüğü arttırıcı faydalarından yararlanılması hedeflenmiştir. Kurkuminin düşük değerlerdeki sudaki çözünürlüğünün polietilenglikol ile geliştirilmesi doğrultusunda çalışmalar yapılmıştır.

Bu çalışmadaki denemelerde,kompleks yapıcı madde olarak kullanılan polietilenglikolün üç farklı çeşidi kullanılmıştır.Üç farklı çeşidin birbirinden farkı molekül ağırlıklarıdır. Polietilenglikolün molekül ağırlığı 200,600 ve 1500 olan üç çeşidi burada kullanılmıştır. Çalışmaları bir esasa dayandırabilmek ve üzerinden hesaplamaları yapabilmek için her üç molekül ağırlığındaki polietilenglikol için belirlenen konsantrasyonlarda kurkumin içeren çözeltiler hazırlandı. Çözeltilerin konsantrasyonları,kalibrasyon doğrusunun doğrusal olabileceği şekilde hesaplandı. Bu çözeltiler için hazırlanan stok çözelti 1 mg kurkumin ile 9 mg polietilenglikol içermekteydi. Bu stok çözelti kullanılarak hazırlanan üç farklı konsantrasyondaki çözeltilerin ultraviolet spektrumdaki absorbansları ölçülerek kaydedildi. Kalibrasyon çözeltilerine ait ölçümler UV Visible dedektör ile 300-500nm dalgaboyu aralğında gerçekleşti.Renkli bir bileşik olduğu için belli bir dalgaboyunda pik vermesi beklenen kurkuminin absorbans değerlerinden yola çıkarakpik verdiği maksimum

dalgaboyu tespit edildi. Kurkumin için belirlenen maksimum dalgaboyu 430 nm olarak belirlendi. Maksimum dalgaboyunda elde edilen absorbans değerleri y eksenine,çözeltilerin konsantrasyonları da x eksenine konularak çizdirdiğimiz grafik sonucunda elde edilen kalibrasyon doğrusu ve bu doğruya ait olan denklem kalibrasyon verilerini oluşturdu.

Kalibrasyon datalarını elde ettikten sonra,polietilenglikol ile kurkumin içeren test çözeltileri hazırlandı. Polietilen ile kurkumini içeren numuneler miktarca %5, %10,%15 ve %20 oranında polietilenglikol içerecek şekilde hazırlandılar. %5 oranında polietilenglikol içeren çözelti için 0.5 g polietilenglikol 10 g suda çözülerek üzerine 20 mg kurkumin eklendi. %10 oranında polietilenglikol içeren çözelti için 1.0 g polietilenglikol 10 g suda çözülerek üzerine 20 mg kurkumin eklendi. %15 oranında polietilenglikol içeren çözelti için 1.5 g polietilenglikol 10 gr suda çözülerek üzerine 20 mg kurkumin eklendi. %20 oranında polietilenglikol içeren çözelti için 2.0 g polietilenglikol 10 g suda çözülerek üzerine 20 mg kurkumin eklendi.Dört farklı orandaki çözelti tüm molekül ağırlığındaki polietilenglikoller için hazırlandı. Polietilenglikol ile kurkumin içeren çözeltiler bir saat boyunca ultrasonik banyoda tutulduktan sonra santrifüj edilerek dekante edildi ve değerlendirildi. Dört farklı oranda polietilenglikol içeren çözelti her bir molekül ağırlığı için üçer kez hazırlanarak çalışmanın en az üç kez tekrar edilen verilere dayanması sağlandı.Hazırlanan tüm çözeltilere ait ölçümler,kalibrasyon çözeltileriyle aynı şartlarda,UV Visible dedektör ile 300-500nm dalgaboyu aralığında gerçekleştirildi. Tüm ölçümlerde maksimum dalgaboyu olan 430 nm'de elde edilen absorbans değerleri kaydedildi. Kalibrasyon grafiklerinden elde edilen doğru denklemleri, Lambert-Beer yasasından yola çıkarak absorbans değerlerini kullanarak madde miktarını hesaplamada kullanıldı. Dolayısıyla maksimum dalgaboyunda elde edilen absorbans değerleri, kalibrasyon grafiklerinden elde edilen doğru denklemlerindeki bilinmeyen yerine konularak çözeltilerde çözünen madde miktarı hesaplandı.

Tüm çalışmalar sonrasında kurkuminin sudaki çözünürlüğününçözeltilerde kullanılan polietilenglikolün molekül ağırlığı arttıkça arttığı gözlemlendi. Ayrıcaaynı molekül ağırlığına sahip polietilenglikol çözeltileri arasında da yüksek oranda polietilenglikol içeren çözeltilerde kurkuminin daha iyi çözündüğü gözlemlenmiştir.Ancak polietilenglikolün molekül ağırlığının 1500 olduğu çözeltilerde, %20 oranında polimer içeren çözeltinin en yüksek kurkumin çözünürlüğüne sahip olması beklenirken, %10 ve %15 oranındaki çözeltilerdeki değerler daha yüksek çıkmıştır.

1 INTRODUCTION

Curcumin is an organic component with a sharp yellow color extracted from the *Curcuma longa* plant. Curcumin exhibits antioxidant, anti-inflammatory, and antimicrobial activities [1, 2]. Curcumin has properties like making the taste of some products better in food industry and it also gives color to some foods used in dietary applications, and it is also used for healing in medicine [1]. Curcumin is normally insoluble in water at acidic or neutral pH values. Curcumin is not completely absorbed; its oral bioavailability is also low. This effect is probably due the poor solubility and slow dissolution. This is the reason that the solubility of the curcumin in water should be increased in some way.

In an attempt to overcome this shortcoming, we have prepared water-soluble curcumin-PEG complexes. Water solubility of curcumin in media containing surfactants was assessed to develop a dissolution system. For this purpose three different types of poly(ethylene glycol) were used in our studies. Curcumin was added to these solutions at different concentrations by sonication. The concentrations of solutions were determined by UV-Vis measurements.

As a result, the solubility of curcumin was increased with PEG complexes. The solubility of curcumin was increased as the amount of PEG was increased in all molecular weights. The aim is to find the optimum PEG and curcumin concentrations which are the lowest PEG and the highest curcumin.

2 SOLUBILITY

The property solubility can be measured by so many techniques and these techniques are commonly used in colligative property.When a solute in a solid form is allowed to get into contact with a solvent, it dissolves.This action is referred as dissolution,but also can be explained as being surrounded by solvent molecules to be transferred in the liquid phase.Dissolution generally continues until the solution becomes saturated. Saturation is a term about the equilibrium of solid form and solvated form in the same solution. In a saturated solution, the chemical potential of the solute in solid form can be shown as $\mu^*_{B(s)}$, and the chemical potential of B in the solution can be shown as μ_B (Figure 2.1).

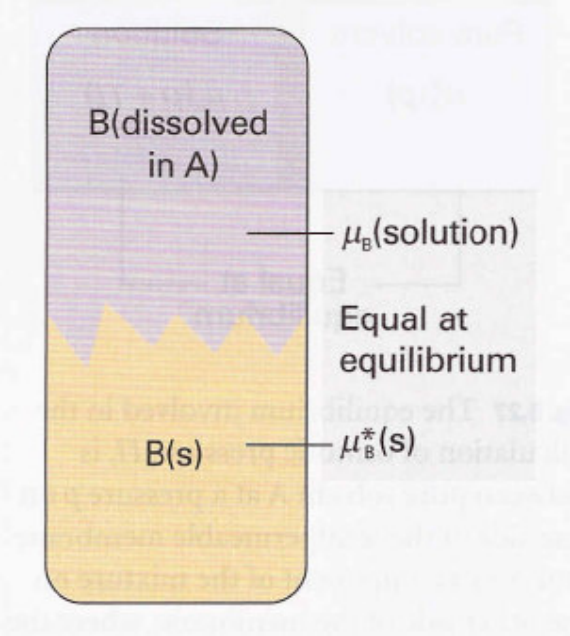

Figure 2.1: The heterogeneous equilibrium involving the calculation of the solubility is between the pure solid and B in the mixture.

Related equations about solubility can be shown as :

$\mu^*_B = \mu_B * (l) + RT \ln XB$

we can write

$\mu^*_B (s) = \mu^*_B (l) + RT \ln XB$

Since :

The equation is plotted in Figure 2.2 .

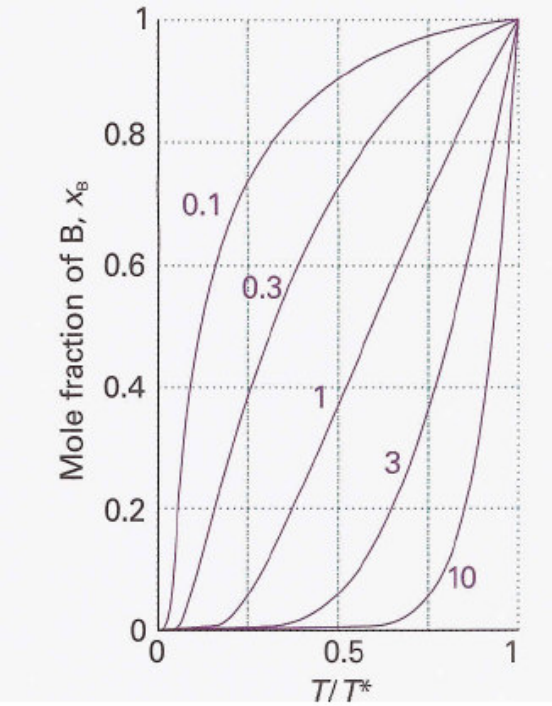

Figure 2.2: The variation of solubility (the mole fraction of solute in a saturated solution) with temperature (T* is the freezing temperature of the solute). Individual curves are labelled with the value of $\Delta H_{fus} / R T^*$.

Figure 2.2 explains that the solubility of B decrases exponentially when the temperature is lowered starting from its melting point. This figure also shows that solutes with high melting points and large melting enthalpies have low solubilities at normal temperatures.

3 SURFACTANTS

3.1 Definition of Surfactans

Surface active agents are usually termed as surfactants. Surfactans are amphipathic molecules, which means that they have a polar end attached to a water molecule and other end repelled to the first. Surfactant molecules mostly consist of a non-polar hydrophobic end, usually a straight or branched hydrocarbon or fluorocarbon chain with 8–18 carbon atoms, which is attached to a polar or ionic hydrophilic end. The hydrophilic end of the surfactant molecule can be nonionic, ionic or zwitterionic and usually accompanied by counter ions. In aqueous media, the interaction between the hydrocarbon and the water molecule is very weak but the interaction between the polar or ionic side and water is strong because of the dipole or ion-dipole interactions. This strong interaction with the water molecules makes the surfactant soluble in water. However, the interaction of dispersion and hydrogen bonding between the water molecules lead to the squeezing of the hydrocarbon chain out of the aqueous phase. These outer hydrocarbon chains are mostly referred to as being hydrophobic. The balance between hydrophobic and hydrophilic parts of the molecule gives these systems some unique properties like becoming collected at various interfaces and association in solution.

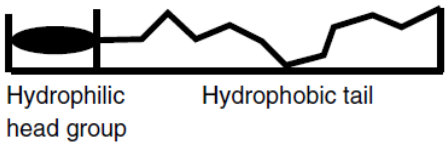

Figure 3.1: The basic molecular structure of a surface-active material, which includes the hydrophobic group having little attraction for water and the hydrophilic group having strong interactions with water.

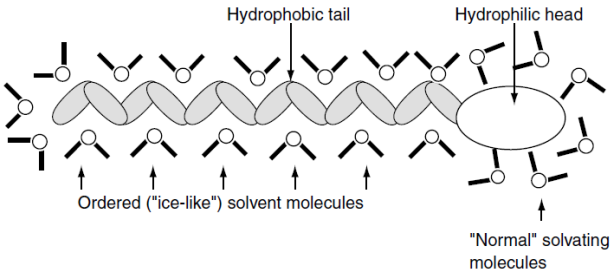

Figure 3.2: For a surfactant molecule in water, the hydrophobic tail will be ‘‘solvated’’ with an ice-like structure of associated solvent molecules. The hydrophilic head will be solvated in the usual way.

The free energy created by the phase boundary has an important affect on the surfactant's adsorption. When this free energy is low, the adsorption of the surfactant will be increased The interfacial free energy per unit area can be explained as the amount of the required work for expanding the interface. This interfacial free energy is also known as surface or interfacial tension, and it can be shown with symbol γ ,and it is given in mJ m^{-2} or mN m^{-1}. Adsorption of the surfactant molecules at the interface lowers the surface tension. The higher the surfactant's adsorption, the larger the reduction in γ. The degree of magnitude of surfactant's adsorption at the interface depends on two parameters, the first being the surfactant structure and the other is the nature of the two phases that meet the interface.

Surface active agents also form micelles via aggregation in solution. The free energy of the system can be decreased by reducing the contact between the hydrocarbon chain and water. The reduction in free energy will lead the micellar formation, also known as micellization. In the micelle, the surfactant's hydrophobic groups are directed towards the interior of the aggregate and the polar head groups are directed towards the solvent. The formed micelles exist in a very dynamic equilibrium. The exchange rate between a surfactant molecule and the micelle may differ from each other by the magnitude parameter, which depends primarily on the structure of the surfactant molecule.

Surfactants are very useful in so many areas in the chemical industry, including detergents, paints, cosmetics, pharmaceuticals, agrochemicals, and plastics. Moreover, surfactants play a major role in the oil industry, such as enhancing the oil's recovery.

3.2 Classification of Surfactants

The easiest classification of surfactants is based on the structure of the molecules. The nature of the solubilizing functionality may depend on the hydrophile in aqueous systems .Along with each classification according to the solubilizing agent, there will be subgroups for the respective surfactants.

Four general groups of surfactants are defined as follows:

1. Anionic, with the hydrophilic group carrying a negative charge such as carboxyl ($RCOO^- M^+$), sulfonate ($RSO_3^-M^+$), or sulfate ($ROSO_3^-M^+$).

2. Cationic, with the hydrophilic entity with a positive charge such as the quaternary ammonium halides ($R_4N^+X^-$).

3. Nonionic, with the hydrophile has no charge but derives its solubility in water from highly polar groups such as polyoxyethylene($-OCH_2CH_2O^-$), sugars or similar groups.

4. Amphoteric, the molecule has either a negative and a positive charge on the chain such as the sulfobetaines.

3.2.1 Nonionic surfactants

3.2.1.1 Poly(ethylene gylcol)

The manufacturing process of poly(ethylene glycol)s includes the polymerization of ethylene oxide with a chosen starting material like water, monoethylene glycol or diethylene glycol. Polymerization goes further under alkaline catalysis. After the desired molecular weight is reached, the reaction can be terminated by neutralizing the catalyst with an acid. The molecular weight is usually checked by viscosity measurements as an in-process control during the experiments. Normally, lactic acid is used, but also acetic acid or other acids can be employed as well. The result is a very simple chemical structure: $HO-[CH_2-CH_2-O]_n-H$.The formulation of this chemical structure tells that n is given as the number of repeating ethylene oxide units. As an abbreviation for polyglycols, the term “PEG” is used, in combination with a numerical value describing the total molecular weight of the polymeric structure.

PEGs with a mean molecular weight up to 400 are nonvolatile liquids at room temperature. PEG 600 has a melting range of from 17 to 22 °C, so it may be in liquid form at room temperature. But PEG 600 is mostly sticky at lower environmental temperatures, while PEGs a mean molecular weight of 800 to 2000 are pasty materials with a low melting range than that of PEG 600. PEGs with molecular weight above 3000 may be found as solids but also as powders. Commercially available polyglycols are also available with a molecular weight up to 35,000. The hardness of polyglycols increases with increasing molecular weight; however, the melting range goes up to a maximum value of about 60 °C . The most important property of all PEGs is their water solubility and it makes them fully adequate for use in many different applications. Molecular weights up to PEG 600 may be combined with water in any desired ratios. Solid PEG molecules have excellent water solubility values. Water solubility of PEGs increases with increasing molar mass, even 50% (w/w) of a PEG 35,000 can be dissolved, at room temperature, in water. One of the important properties about PEGs is that regarding their nonionic structure, their solubility and viscosity properties are not affected by the electrolytes in the media. PEGs are quite soluble in hard water or in other aqueous solutions of various salts.

Another very important property of PEGs is their ability to solvate numerous substances that are hardly soluble in water. This action can be explained as the formation of a complex between the polyethylene glycol molecule and the desired active substance.

Polyethylene glycols have important properties of toxicological safety like dermal compatibility, which is supported by absorptive investigations. Polyethylene glycols also have been used for many years in cosmetics, foodstuffs, and the pharmaceutical industry and are registered in all relevant pharmacopeias. The acceptable daily intake for polyethylene glycols in foodstuffs is defined by World Health Organization (WHO) as a maximum of 10 mg/kg bodily weight [6].

4 ANTIOXIDANTS

4.1 General Information

Free radicals are highly reactive chemicals which may damage the bodily cells of the human being. When an atom or a molecule gains or loses a negative charge, that is an electron, a phenomenon called the free radical formation occurs. Free radicals play an important role in the human body, in which they are naturally formed [1, 2]. Free radicals may also be very hazardous in especially high concentrations and damage the human body cell and its main components including DNA, proteins, and cellular membranes. Damage caused by the free radicals to the DNA may also be encountered in cancer and other diseases [1, 2].

Free radicals with high concentrations may cause exposure to ionizing radiation and other environmental toxins in the human body. When ionizing radiation occurs in an atom or in a molecule in human cell, an electron may be lost. This electron lost will support free radical formation. The production of abnormally high levels of free radicals is the mechanism by which ionizing radiation kills cells.Additionally, some toxins including smoking cigarette, some metals, and high-oxygen atmospheres, may contain large amounts of free radicals or lead the bodily cells to produce more free radicals.

4.1.1 Antioxidants

Antioxidants may be defined as chemicals which interacts with free radicals and neutralize them for protecting human body cells. Another term used for antioxidants is free radical scavengers.

The human body makes some of the antioxidantsnaturally and these antioxidants neutralize theharmful free radicals. These antioxidants are called endogenous antioxidants. Despite the antioxidants produced by the human body,the human body mostly needs the external antioxidants. These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants. Some dietary supplements include dietary antioxidants also.[1, 3].

Beta-carotene, lycopene, and vitamins A, C, and E (alpha-tocopherol) are some spesific examples of dietary antioxidants. Selenium,a mineral element is also considered as a dietary antioxidant because the proteins,which have selenium as an essential compond,shows some antioxidant effects[4].

Curcuminoids have been consumed as therapeutic medicins over the centuries. In the alternative Ayurvedic medicine, curcumin is a common treatment for various health conditions such as, asthma, bronchial hyperactivity and allergy, as well as for hepatic disorders, anorexia, rheumatism, diabetic wounds, runny nose, cough, and sinusitis Curcumin has also been used for the treatment of some parasites and as a cure for poisoning, snakebites and various other complaints [9].

4.2 Properties of Curcumin

Curcumin can be obtained by extracting the turmeric,which is the ground rhizomes of *Curcuma longa L.* Curcumin can be purified by crystallization [9]. The origin of the plant *Curcuma longa L.*, which belongs to Zingiberaceae family, is India. The plant is distributed throughout tropical and subtropical regions of the world, being widely popular especially in southeast Asian countries. Turmeric, i.e., the ground rhizomes of *Curcuma longa L.*, has a long history about being used in food as a spice.The main use of curcumin is as an ingredient in many varieties of curry powders and sauces, where curcumin from turmeric is a main coloring substance and gives a sharp yellow color to the food.

More recently, it has been used by the food industry as additive, flavoring, preservative, and coloring agent (e.g., in mustard, margarine, soft drinks, and beverages). Curcumin is also listed in the international numbering system for food additives with the code E100 since it is considered as a safe coloring agent. Turmeric is also used in non-medical applications cosmetics, spesifically in Hindu rituals and ceremonies. Turmeric is also commercially available,whereas these commercial products may contain essential oils, polyphenols, protein, fat, minerals, carbohydrates, and moisture. The aromatic properties of turmeric are linked to its volatile essential oils.

For centuries,turmeric has also been used to treat many disorders including rheumatism, bodily aches, dermal diseases, wounds, intestinal worms, , intermittent

fevers, hepatic disorders, biliousness, urinary discharges, dyspepsia, inflammation, constipation, leukoderma, amenorrhoea, and colic inflammation [10].

The color of turmeric is yellow because it has polyphenolic curcuminoids, which constitute approximately 3% to 5% of most turmeric preparations. The alcoholic extract of turmeric mainly contains three curcuminoids, namely curcumin (curcumin I), desmethoxycurcumin (curcumin II), and bisdesmethoxycurcumin (curcumin III).

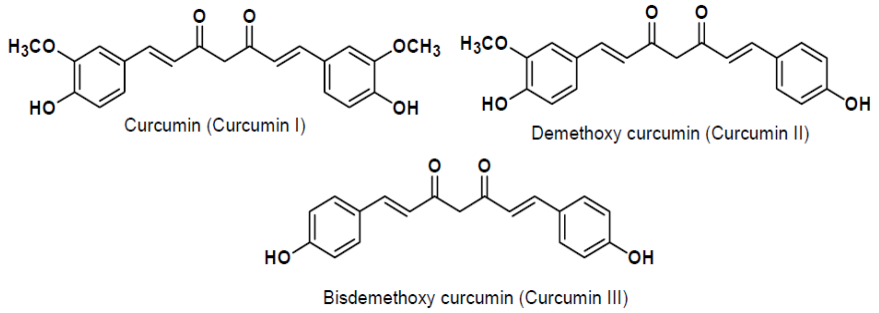

Figure 4.1: Structure of three major curcuminoids in turmeric.

The chemical name of curcumin is (E,E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5 dione) is a bis-α,β-unsaturated β-diketone. It has a molecular weight of 368.38 Da, a melting point of 179–183 °C, and a chemical formula of C21H20O6. Under physiological conditions, curcumin can exist in both an enol and a bis-keto form, which is in an equilibrium.

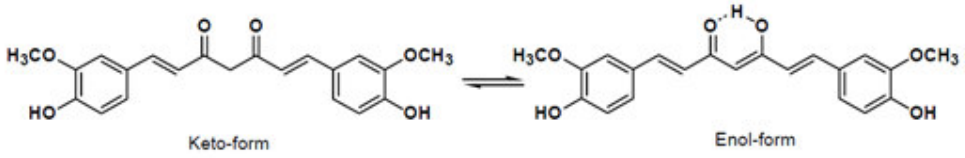

Figure 4.2: pH-dependent keto- and enol- tautomeric form of curcumin.

The solubility of curcumin in dimethylsulfoxide (DMSO), ethanol or acetone is well enough, but it is sparingly soluble in water. If curcumin is an acidic media,the keto form predominates and curcumin acts as a potent donor of H-atoms. In contrast, under alkaline conditions (≥pH 8), the enolic form predominates, and the phenolic part of the molecule plays the role as an electron donor . In solution, it has been demonstrated that 90% of curcumin was found to degrade to trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexanal,vanillin, feruloylmethane, and ferulic acid within 30 minutes. Curcumin is similarly unstable at basic pH values, but in the

presence of calf serum or human blood, less than 20% of curcumin was found to decompose in 1 hour. Addition of antioxidants (ascorbic acid, N-acetylcysteine or glutathione) to culture media was also shown to inhibit the degradation of curcumin. Yellow curcumin changes to dark red color at alkaline pH and under physiological conditions; the λmax for curcumin is observed at 420 nm [9].

5 EXPERIMENTAL PART

5.1 Apparatus and Chemicals

Apparatus: Shimadzu Double-Beam UV-150-02 Spectrophotometer, Sonorex RK 100H Ultrasound System, Precisa 205A electronic balance, Millipore MilliQ UV Water Purification System, Magnetic Stirrer, Centrifuge.

Other equipment: Thermometer, Beakers, Volumetric flasks, Pasteur pipettes, Quartz cells.

Chemicals: Deionized water, polyethylene glycol, curcumin.

5.2 Washing and Cleaning Procedure

Cleaning is very important in a chemical experiment. During the experiments the following washing and cleaning procedure is performed:

Firstly, the excess impurities and chemicals in an apparatus are poured to the waste bottle and the apparatus is cleaned with water and dish soap by gentle brushing. In order to clean away the inorganic impurities, the apparatus is treated by a base solution and for organic impurities an acid solution is used. Then it is washed with water again and put in constant temperature oven for drying.

The preparation of chromic acid solution: The critical point while preparing the acid solution is pouring the water first and then acid on it. 5 mL water is poured into a beaker and then 2 g of potassium dichromate is added. When the chromate is dissolved completely, 40 mL of sulfuric acid solution is added and the chromic acid solution is obtained.

5.3 Procedure

5.3.1 Preparation of calibration solutions

In order to determine the concentration and wavelength range, curcumin solutions were prepared with different molecular weights of curcumin :

- Matrix solution : 1 g of PEG was weighed and dissolved in 9 g of water .
- C_1 : 5 g of matrix solution and 1,0 mg curcumin were mixed and dissolved
- C_2 :2 g of matrix solution and solution 1 were mixed and dissolved
- C_3 : 3 g of matrix solution and solution 2 were mixed and dissolved

The concentrations of calibration solutions are calculated as follows :

- C_1 = 0.046 mg/g
- C_2 = 0.032 mg/g
- C_3 = 0.023 mg/g

UV absorbance of these solutions were measured in a range of 300 nm to 500 nm by Shimadzu Double-Beam UV-150-02 Spectrophotometer and maximum absorbance wavelength was determined as 430 nm.

Lambert-Beer Law:

$A = \varepsilon b c$

Where;

A is molar absorbance (no units)

ε is the molar absorbtivity (L mol^{-1} cm^{-1})

b is the length of the sample (1 cm)

c is the concentration of the compound in solution (mol L^{-1})

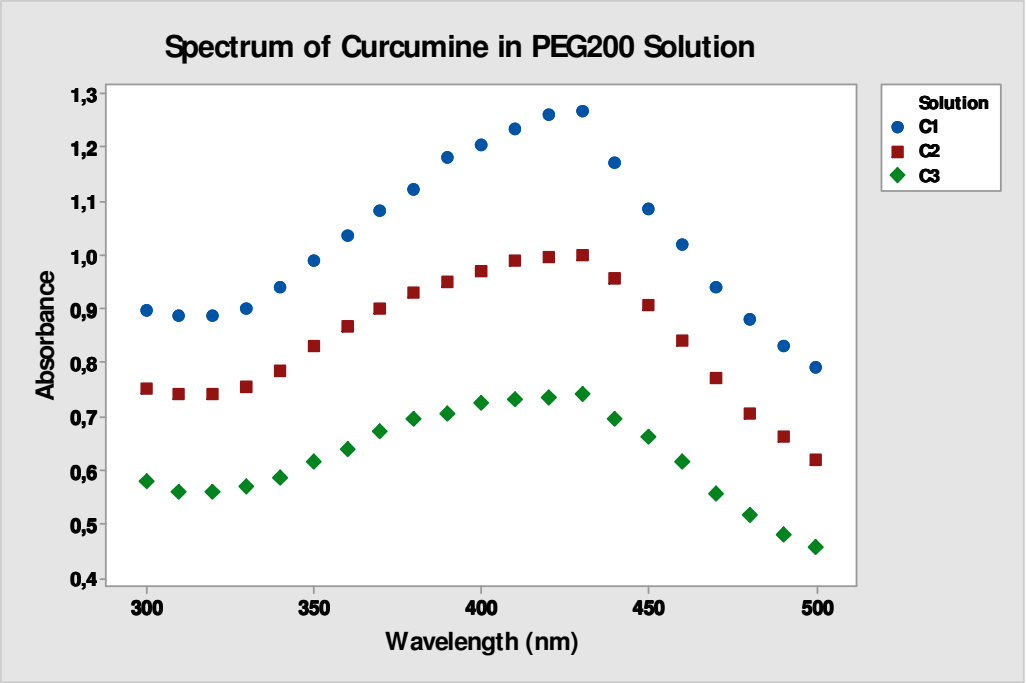

Figure 5.1: Spectrum of Curcumin in PEG200 Solution.

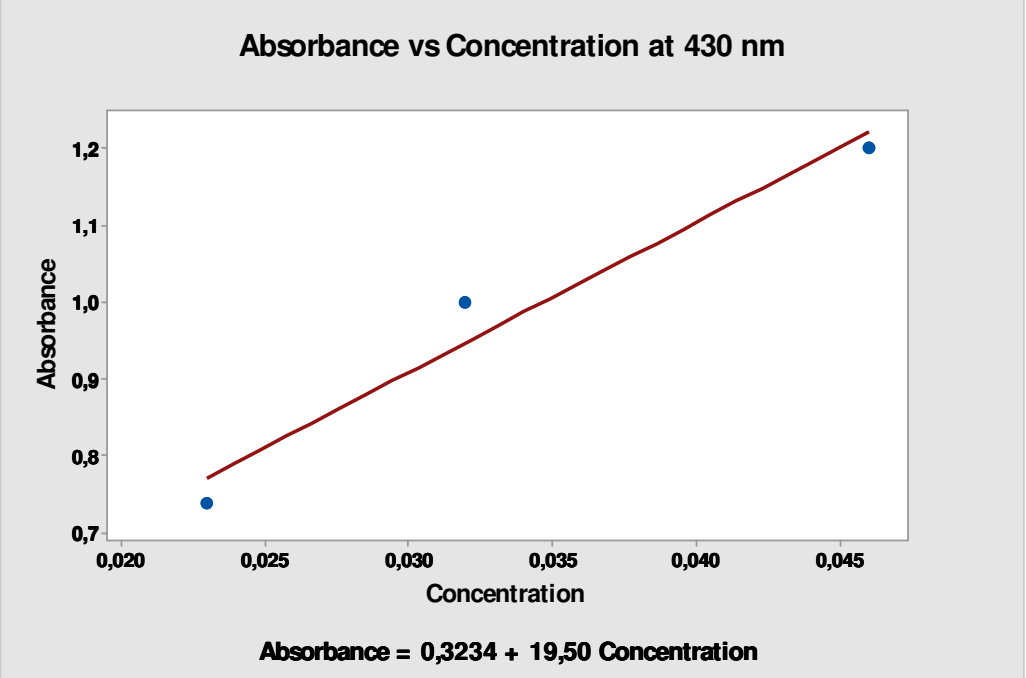

Figure 5.2: Calibration Curve for PEG 200.

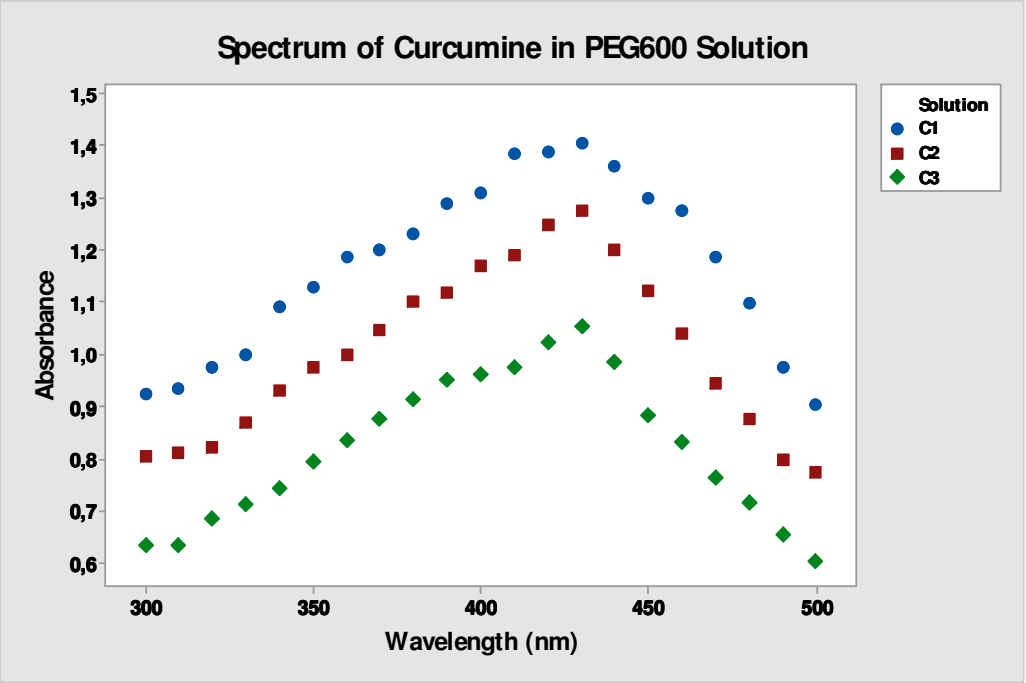

Figure 5.3: Spectrum of Curcumin in PEG600 Solution.

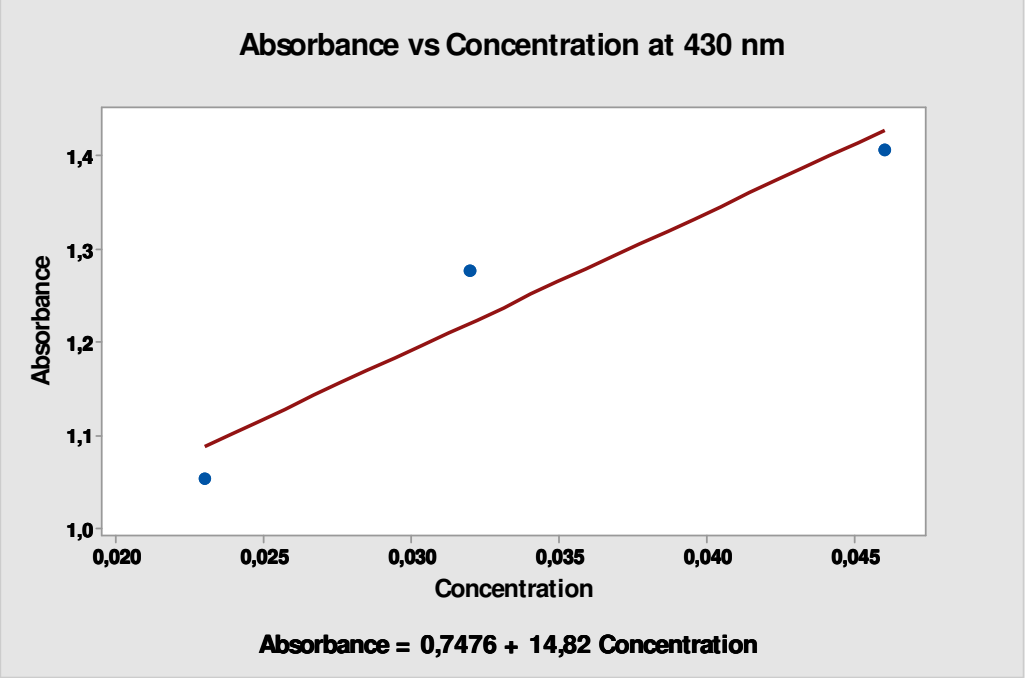

Figure 5.4: Calibration Curve for PEG 600.

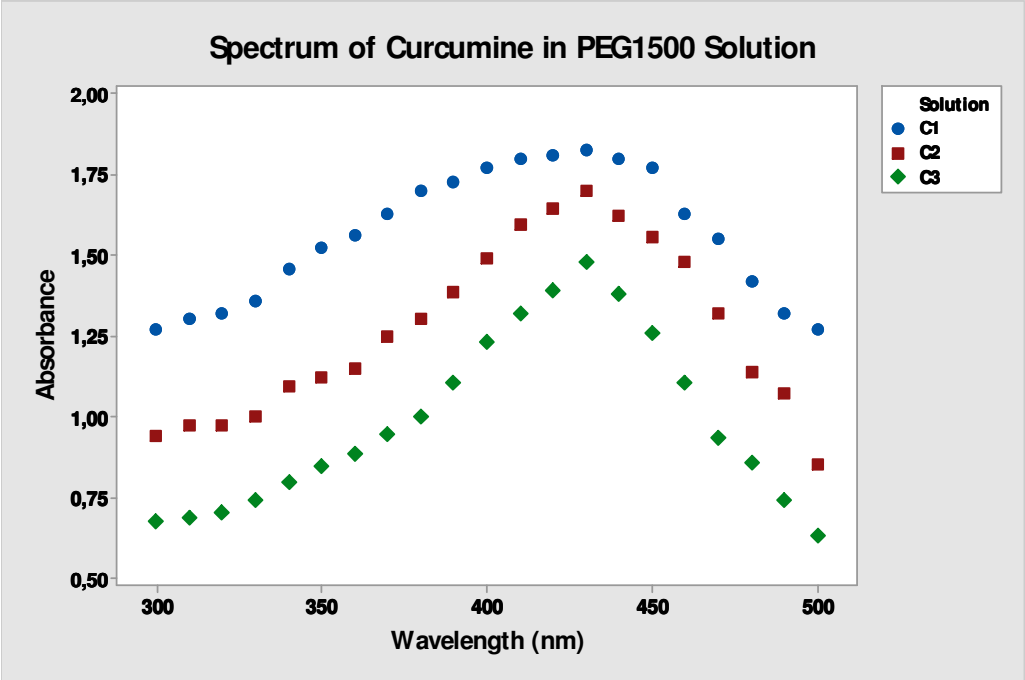

Figure 5.5: Spectrum of Curcumin in PEG1500 Solution.

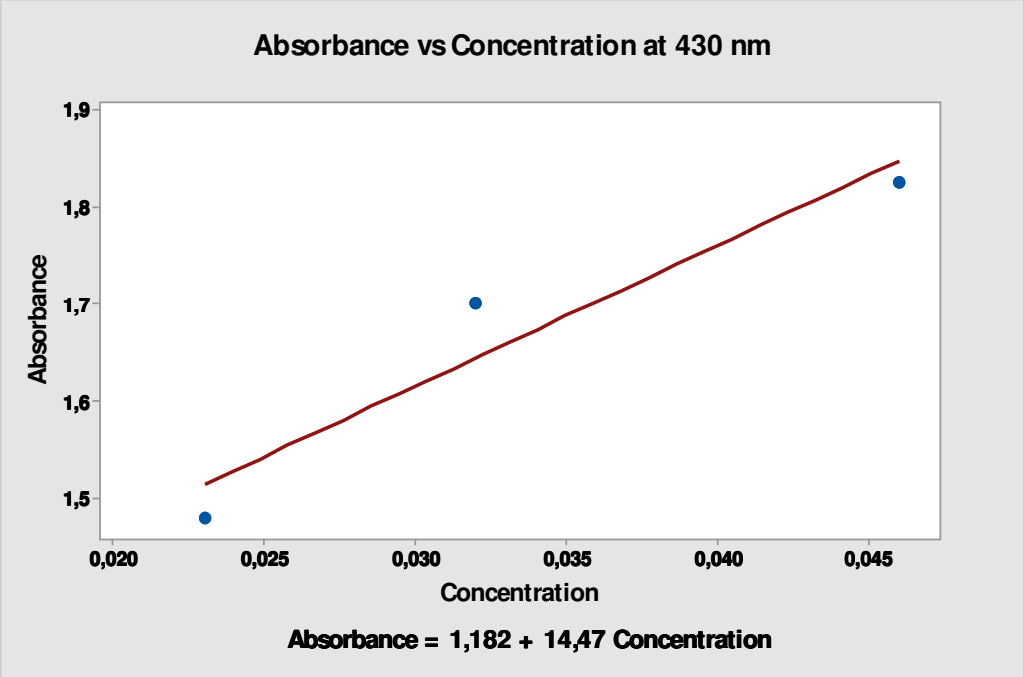

Figure 5.6: Calibration Curve for PEG 1500.

5.3.2 Preparation of Sample Solutions

5.3.2.1 Preparation of PEG 200 Solutions

Four solutions with different concentrations are prepared.

0.5, 1.0, 1.5, and 2.0 g of PEG 200 were dissolved in 10 g of water, respectively.

20 mg of curcumin was weighed and added to all solutions. Then, the solutions were sonicated for an hour at 20˚C. The solutions were then centrifugated for thirty minutes with 6000 revolutions per minute. At last, centrifugated solutions were decanted.

5.3.2.2 Preparation of PEG 600 Solutions

0.5, 1.0, 1.5, and 2.0 g of PEG 600 were dissolved in 10 g of water, respectively.

20 mg of curcumin was added to all solutions. The solutions were sonicated for an hour at 20˚C. Then, the solutions were centrifugated for thirty minutes with 6000 revolutions per minute. At last, centrifugated solutions were decanted.

5.3.2.3 Preparation of PEG 1500 solutions

0.5, 1.0, 1.5, and 2.0 g of PEG 1500 were dissolved in 10 g of water, respectively.

20 mg of curcumin was weighed and added to all solutions. Then, the solutions were sonicated for an hour at 20˚C. The solutions were then centrifugated for thirty minutes with 6000 revolutions per minute. At last, centrifugated solutions were decanted.

UV spectrum of each solutions recorded and curcumin amount was calculated from the maximum absorbance values at 430nm.

6 RESULTS AND DISCUSSION

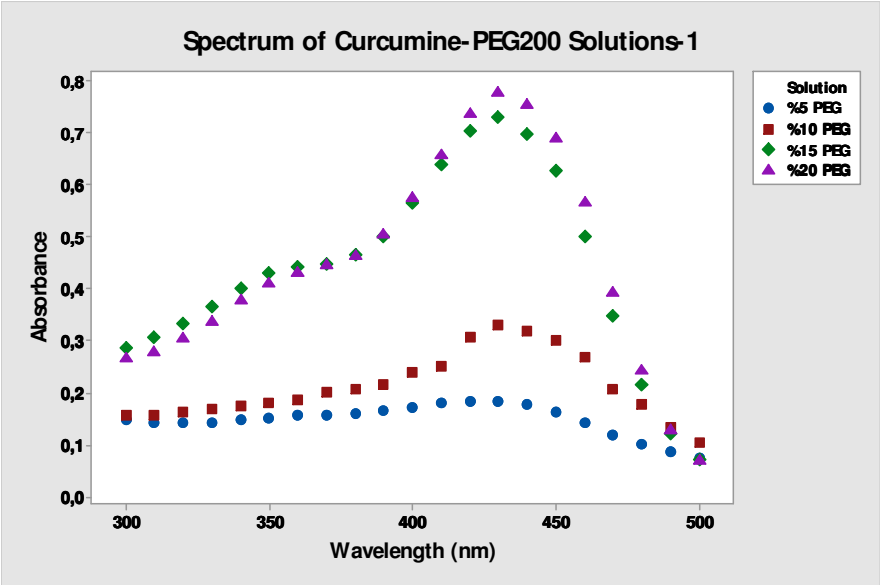

Figure 6.1: Spectrum of Curcumin-PEG200 Solutions, first group.

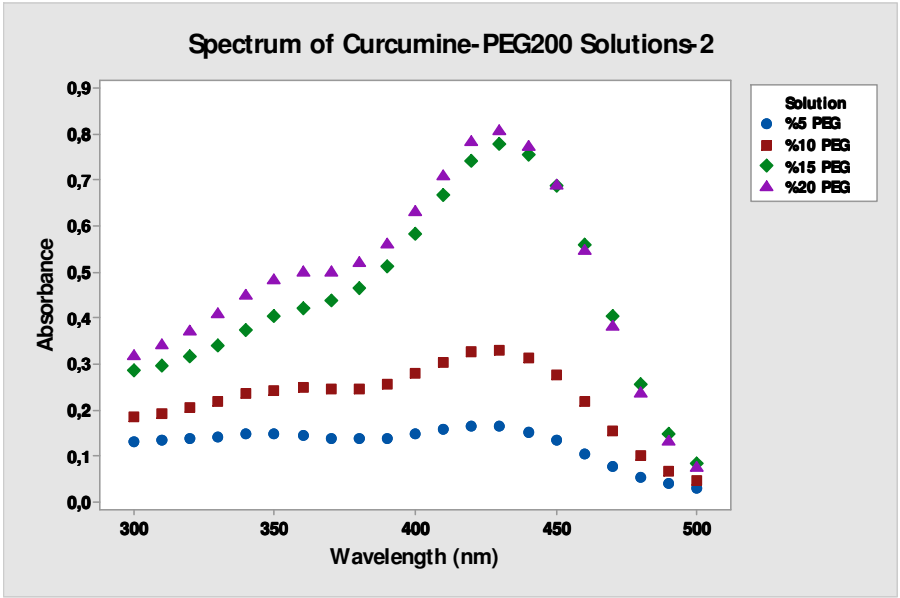

Figure 6.2: Spectrum of Curcumin-PEG200 Solutions, second group.

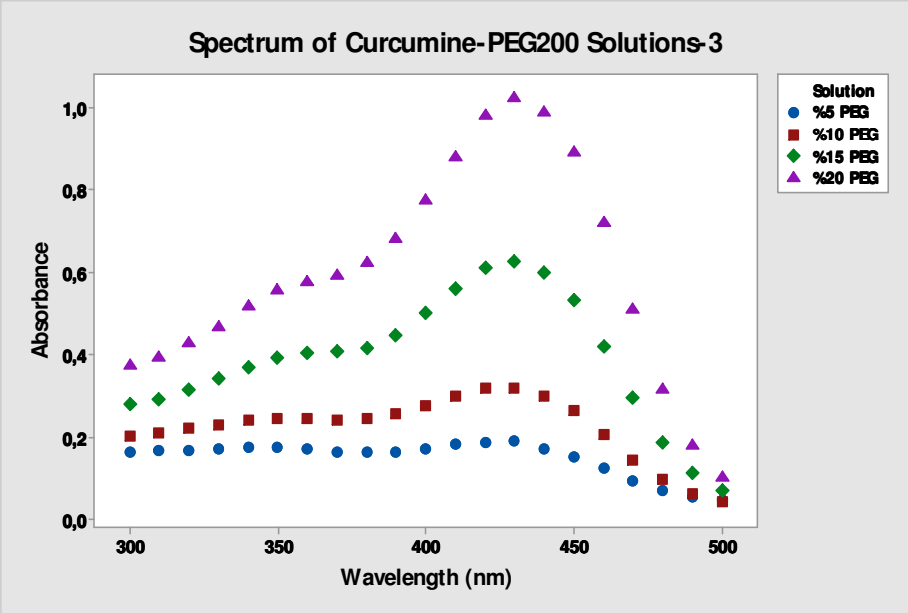

Figure 6.3: Spectrum of Curcumin-PEG200 Solutions, third group.

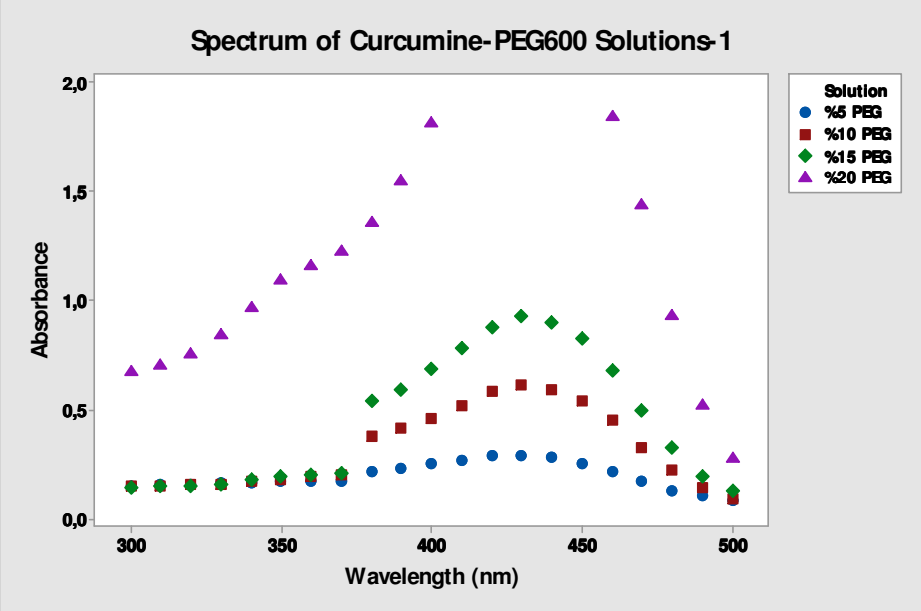

Figure 6.4: Spectrum of Curcumin-PEG600 Solutions, first group.

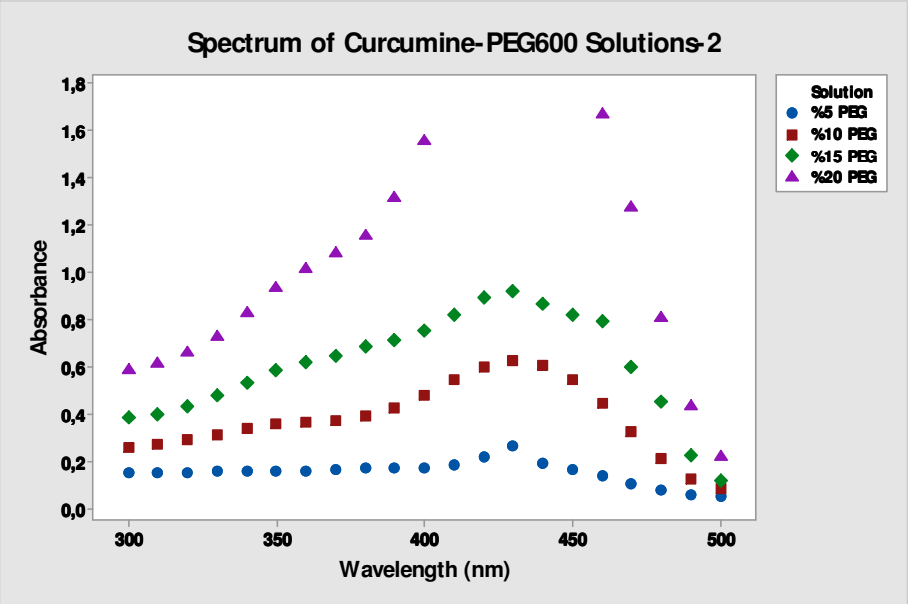

Figure 6.5: Spectrum of Curcumin-PEG600 Solutions, second group.

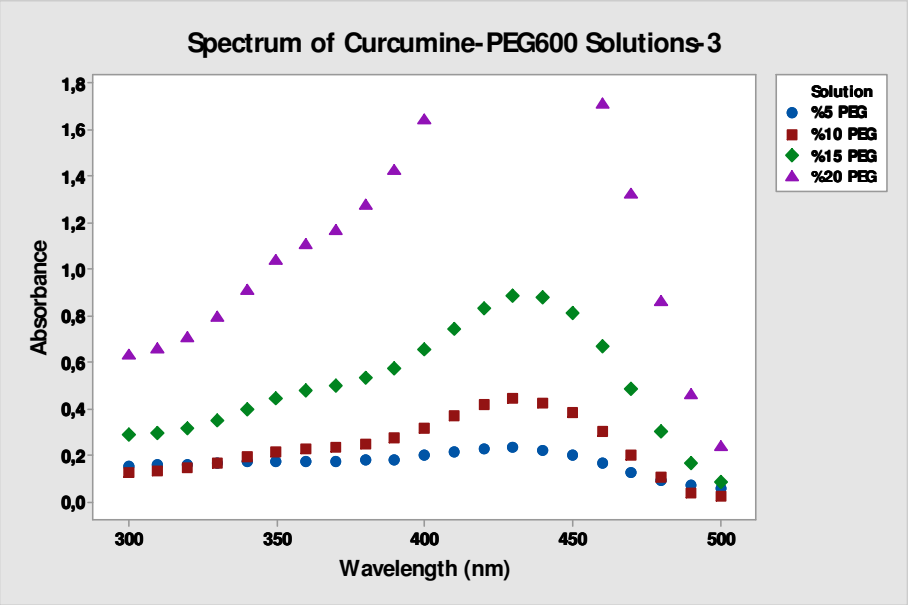

Figure 6.6: Spectrum of Curcumin-PEG600 Solutions, third group.

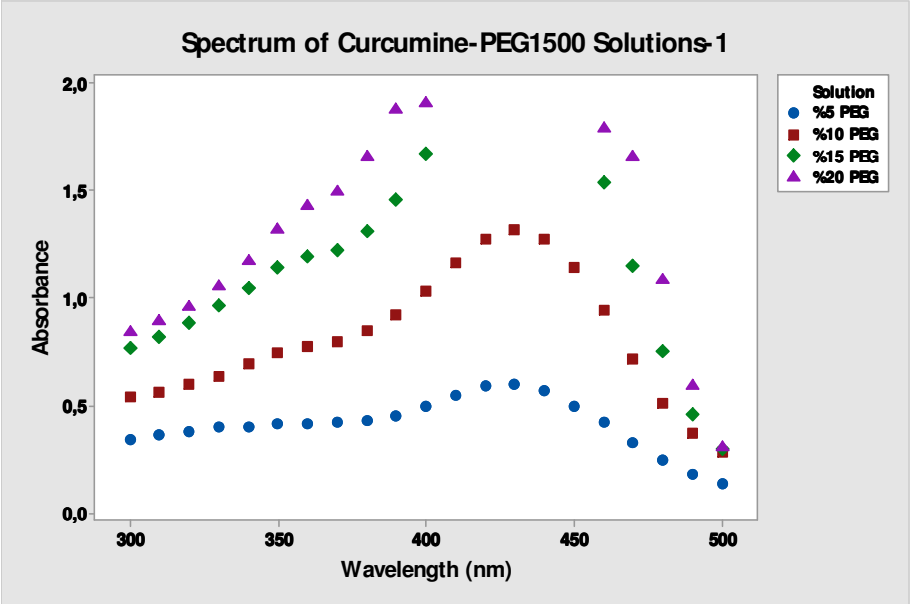

Figure 6.7: Spectrum of Curcumin-PEG1500 Solutions, first group.

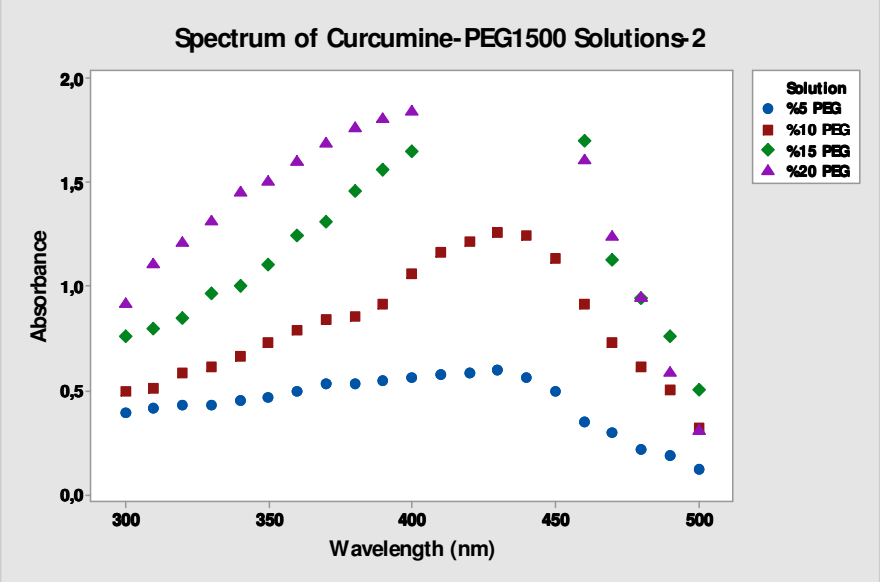

Figure 6.8: Spectrum of Curcumin-PEG1500 Solutions, second group.

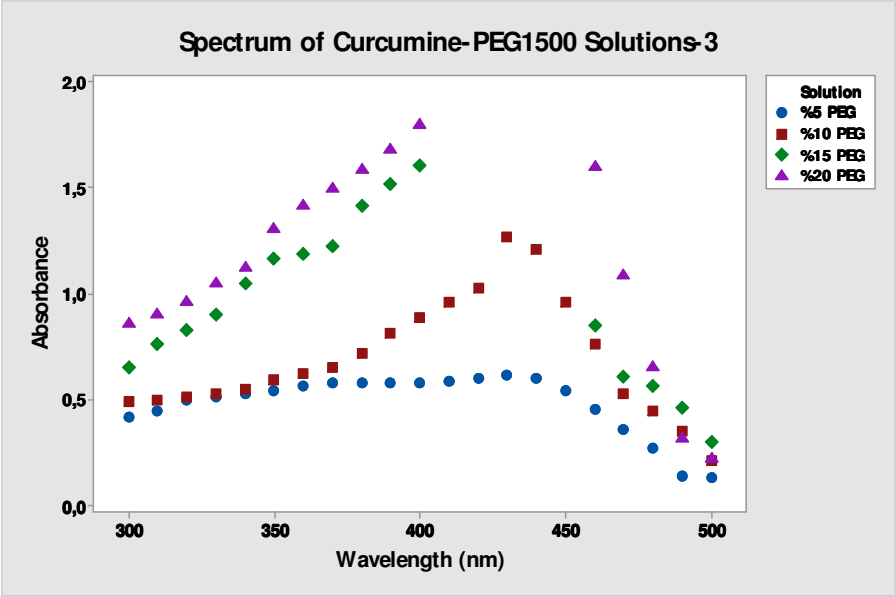

Figure 6.9: Spectrum of Curcumin-PEG1500 Solutions, third group.

Table 6.1 : Curcumin amount in PEG solutions.

	Curcumin amount in PEG solutions (mg curcumin/10 g PEG solution)		
PEG%	**PEG200**	**PEG600**	**PEG1500**
5	3,9	5,1	9,8
	3,5	4,7	9,8
	4,0	4,2	10,1
0	6,8	9,8	20,0
	6,7	10,0	19,5
	6,5	7,3	19,5
15	14,5	14,4	**n/a***
	15,5	14,4	**n/a**
	12,5	13,9	**n/a**
20	15,4	**n/a**	**n/a**
	16,0	**n/a**	**n/a**
	20,0	**n/a**	**n/a**

As we can see from Table 6.1 curcumin amounts in the PEG solutions were increased by increasing the PEG molecular weight and concentration. The highest solubility is observed in PEG1500 solutions.

Table 6.2: Solubilization Yield of Curcumin in PEG Solutions.

PEG%	**CurcuminSolubilization Yield in PEG200**	**CurcuminSolubilization Yield in PEG600**	**CurcuminSolubilization Yield in PEG1500**
5	19,1	23,2	49,6
10	33,4	45,1	98,7
15	70,9	71,1	**n/a***
20	86,2	**n/a**	**n/a**

***n/a**: not applicable

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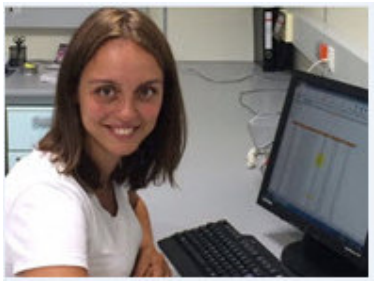
CURRICULUM VITAE



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